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Characterization of 3 strains of *Yersinia pestis*

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Abstract

We have characterized 3 strains of *Yersinia pestis* currently held in the culture collection at DRES by colonial morphology, antibiotic sensitivity and Biolog™ metabolic identification profiles. On agar medium, colonies were round and waxy, resembling other Gram-negative cells such as *Escherichia coli*. Cultures grew better on *Yersinia* selective media than on trypticase soy agar or nutrient agar. Antibiotic sensitivities showed that the 3 strains were sensitive to aminoglycosides, the cephalosporins/ cephams, most of the beta lactams/penicillins (e.g. ampicillin) and quinolones (e.g. ciprofloxacin). These strains were resistant to lincosamide (e.g. clindamycin), macrolides (e.g. erthromycin, colisitin, rifampin and vancomycin) polymyxin B, sulfonamide (e.g. sulfisoxazole) and *Yersinia pestis* strain GB was resistant to doxycycline. This study is part of a larger effort to characterize all the Risk Group 3 bacterial agents used in the research program at DRES. Although DRES does not currently have a mandate to carry out rapid BW agent identification, this and similar studies could help form the basis on which to build such a capability. The concept of a Defence Reference Center for Infectious Agents (DRCIA) is discussed.

Résumé

Nous avons caractérisé 3 souches de *Yersinia pestis* conservées actuellement dans la collection de cultures du CRDS par la morphologie des colonies, la sensibilité aux antibiotiques et les profils d'identification Biolog™. Sur milieu gélosé, les colonies sont rondes et crreuses, semblables celles d'autres bactéries Gram négatives comme *Escherichia coli*. La croissance est meilleure sur des milieux sélectifs pour *Yersinia* que sur gélose trypticase soja ou sur gélose nutritive. Le profil de sensibilité aux antibiotiques montre que les 3 souches sont sensibles aux amynoglycosides, aux céphalosporines/céphames, la plupart des bêta-lactamines/pénicillines (p. ex. ampicilline) et aux quinolones (p. ex. ciprofloxacine). Ces souches sont résistantes à la lincosamide (p. ex. clindamycine), aux macrolides (p. ex. érythromycine, colistine, rifampicine et vancomycine), à la polymixine B, aux sulfamides (p. ex. sulfisoxazole), et la souche GB de *Yersinia pestis* est résistante à la doxycycline. La présente étude fait partie d'un grand projet de caractérisation de tous les agents bactériens appartenant au Groupe de risque 3 dans le cadre du programme de recherche du CRDS. Le CRDS n'a pas pour le moment le mandat d'effectuer une identification rapide des agents de guerre biologique, mais cette étude et des études similaires pourraient faciliter la mise au point d'une épreuve à cet effet. On discute du concept de centre de référence pour la défense contre les agents infectieux (DRDAI).

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Executive summary

Yersinia pestis, is a moderately sized, gram negative bacillus and is the causative agent of plague. As a disease, plague is one of the most notorious of the potential biological warfare agents. In man, it may appear in either the "bubonic" or less frequently in the "pneumonic" form. Bubonic plague is well known in history, including the 14th century outbreak of what is referred to as the "black death" which killed approximately 30% of the population of Europe. Unlike smallpox, which was eradicated through a world wide immunization program in the 1970's, plague outbreaks continue to this day. In 1994 one such outbreak resulted in several hundred deaths in India.

Y. pestis has great potential as both a military and terrorist biological threat agent. *Y. pestis* is used in defence research at DRES as a model system in the study of medical countermeasures, identification and decontamination studies. As part of our safety protocols we have undertaken to characterize the antibiotic sensitivities of *Y. pestis*, as well as the other Risk Group 3 bacterial agents at DRES. This ensures that this information will be quickly available in the event of accidental exposure at DRES, and available to the Canadian Forces (CF) and other government agencies in the event of military or terrorist use. We are also in the process of establishing an identification capability for Risk Group 3 agents of interest to the CF as part of the proposed Defence Reference Center for Infectious Agents (DRCIA).

Biolog™ metabolic profiles were determined for each of the three *Y. pestis* strains held at DRES. These profiles will serve as a key part of planned DRCIA identification capabilities. Antibiotic sensitivity profiles confirm that all *Y. pestis* strains at DRES are sensitive to a variety of antibiotics (including Ciprofloxacin), although we were surprised to find that *Y. pestis* GB strain was resistant to Doxycyclin. It is our intention to aquire additional strains of *Y. pestis* to expand our database profile.

This study is part of a larger effort to characterize all the Risk Group 3 bacterial agents used in the research program at DRES. Although DRES does not currently have a mandate to carry out rapid BW agent identification, this and similar studies could help form the basis on which to build such a capability. The concept of a Defence Reference Center for Infectious Agents (DRCIA) is discussed.

Koumikakis, B.; Bateman, C.; Cherwonogrodzky, J.W. 2000. Characterization of 3 strains of *Yersinia pestis*. TM-2000-164 Defence Research Establishment Suffield.

Sommaire

Yersinia pestis est un bacille Gram négatif de taille moyenne qui est l'agent pathogène de la peste. L'agent de la peste est l'un des agents potentiels de guerre biologique les mieux connus. Chez l'humain, on peut observer la forme « bubonique » ou moins fréquemment la forme « pulmonaire ». La peste bubonique est bien connue dans l'histoire; mentionnons l'épidémie de peste bubonique, également connue sous le nom de peste noire, qui a sévi au XIV^{e} siècle et qui a décimé environ 30 % de la population en Europe. Contrairement à la variole, qui a été éradiquée grâce à un programme d'immunisation mondiale dans les années 1970, la peste fait encore des ravages de nos jours. En 1994, une épidémie de peste a fait plusieurs centaines de victimes en Inde.

Y. pestis est l'un des agents dont l'utilisation sur le plan militaire et dans le contexte du terrorisme biologique présente le plus de possibilités. *Y. pestis* est utilisé en recherche de défense au CRDS comme système modèle dans l'étude des mesures de prévention médicales, de l'identification et dans les études de décontamination. Dans le cadre de nos protocoles de sécurité, nous avons entrepris de caractérisé la sensibilité aux antibiotiques de *Y. pestis* ainsi que celle d'autres agents bactériens appartenant au Groupe de risque 3 conservés au CRDS. Ainsi, ces données seront facilement accessibles dans l'éventualité d'une exposition accidentelle au CRDS, et les Forces canadiennes (FC) et les autres organismes gouvernementaux pourront également les consulter en cas d'utilisation militaire ou terroriste. Nous sommes également en train d'établir un système d'identification des agents du Groupe de risque 3 qui intéressera les FC dans le cadre d'un projet de centre de référence pour la défense contre les agents infectieux (CRDAI).

Des profils métaboliques Biolog™ ont été déterminés pour chacune des trois souches de *Y. pestis* conservées au CRDS. Ces profils constitueront un élément clé du système d'identification du CRDAI prévu. Les profils de sensibilité aux antibiotiques confirment que toutes les souches de *Y. pestis* du CRDS sont sensibles à divers antibiotiques (notamment la ciprofloxaxine), mais nous avons été surpris de constater que la souche GB de *Y. pestis* est résistante à la doxycycline. Nous avons l'intention d'acquérir d'autres souches de *Y. pestis* pour augmenter notre base de données.

La présente étude fait partie d'un grand projet de caractérisation de tous les agents bactériens appartenant au Groupe de risque 3 dans le cadre du programme de recherche du CRDS. Le CRDS n'a pas pour le moment le mandat d'effectuer une identification rapide des agents de guerre biologique, mais cette étude et des études similaires pourraient faciliter la mise au point d'une épreuve à cet effet. On discute du concept de centre de référence pour la défense contre les agents infectieux (DRDAI).

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Introduction

Yersinia pestis, is a moderately sized gram negative bacillus and is the causative agent of plague. As a disease, plague is one of the most notorious of the potential biological warfare agents. In man, it may appear in either the "bubonic" or less frequently in the "pneumonic" form. Over the past 1500 years there have been three pandemics of plague (6^{th}-8^{th} century A.D.; 14^{th} to 17^{th} century A.D. and in the 19^{th}-20^{th} century A.D.) [1]. Most notable was the 14^{th} century outbreak of what was referred to as the "black death" which killed approximately 30% of the population of Europe [2]. Unlike smallpox, which was eradicated through a world-wide immunization program in the 1970's, plague outbreaks continue to this day. Outbreaks in Tanzania (1991) [3] and India (1994) [4] resulted in several hundred cases and many deaths. Sporadic outbreaks occur in western nations as well, such as the US [5].

As a potential biological warfare agent *Y. pestis* has a surprisingly long history. One of the more interesting examples occured in 1346 at the siege of Kaffa. After 3 years of an unsuccessful siege on the Crimean seaport of Kaffa (now called Feodosija) the tartars began using catapults to hurl plague infected corpses over the city walls. People in the city became ill and began to panic and flee. It was reported that only 10 of 1000 refugees in one group survived. This group and others from Kaffa carried the plague to Sicily, Sardinia, Corsica, and Genoa. It may be a coincidence but around this time plague spread throughout Italy and then into Europe. The end result was the "black death" which from 1348 to 1350 killed an estimated 25 million people in Europe, or 30% of the population [2]. Plague was also of interest to the Japanese BW program and was used in attacks against China during the second world war [2].

Y. pestis has great potential as both a military and terrorist biological threat agent. *Y. pestis* is used in defence research at DRES as a model system in the study of medical countermeasures, identification and decontamination studies. As a potential terrorist weapon, *Y. pestis* has not received as much attention as *Bacillus anthracis*. Despite this there was a well publicized incident in which a disgruntled employee at a public health laboratory in the United States (with links to the Aryan Nations movement) acquired a culture of *Y. pestis* from the American Type Culture Collection under false pretenses. Fortunately this employee was arrested and the cultures were seized before they could be used [6].

As part of our safety protocols we have undertaken to characterize the antibiotic sensitivities of *Y. pestis*. This will ensure that this information will be quickly available in the event of accidental exposure at DRES, and available to the Canadian Forces (CF) and other government agencies in the event of military or terrorist use. This study is part of a larger effort to characterize all the Risk Group 3 bacterial agents used in the research program at DRES. Although DRES does not currently have a mandate to carry out rapid BW agent identification, this and similar studies could help form the basis on which to build such a capability. In this report, we present our findings on the culture, antibiotic sensitivities, and Biolog™ metabolic profiles of three strains of *Y pestis*.

Materials and Methods

Culture Strains

Yersinia pestis strains C12 and C092 were acquired from USAMRIID [7](Ft. Detrick Maryland USA) and strain GB was acquired from CBDE Porton Down (Salisbury, UK). Stocks were stored at –70°C either in vials with 25 Protect Beads™ (TSC Ltd., UK) or in 0.9 mL trypticase soy broth with 0.1 ml glycerol Stocks were subcultured onto commercial plates of trypticase soy agar supplemented with 5% sheep red blood cells (Blood agar plates or BAP)(PML Microbiologicals, Wilsonville, OR, US) and grown at 37 °C, 5% CO_2, 90% humidity. For the Biolog™ assay, strains were subcultured onto BUG™ (Biolog Universal growth) medium supplemented with 5% sheep blood (Biolog, Haywood, California). Other media tested were trypticase soy agar (Difco, Detroit, Michigan) and *Yersinia* selective agar (CIN) base with *Yersinia* selective supplement (both from BDH Inc., Toronto, Ontario). All work with these strains was carried out in the DRES BL-3 facility.

Biolog™ Metabolic Identification Profiles

Procedures used were as noted in the Biolog™ Manual or as recommended by personal communications with the staff at Biolog™. Cultures were first subcultured 3 times on Biolog Universal Growth Medium with Blood (BUG+b) and then for the fourth subculture that was to be used for the metabolic profiles these were inoculated onto 3 plates and incubated for 24 hours. Lone colonies or cells from the neighbouring quadrant on the plates were scraped with a sterile toothpick and suspended in Biolog™ diluent to the optical absorbance recommended. *Y. pestis* did tend to clump and settle and so the suspensions were mixed vigorously and dispensed immediately into the GN-2 96-well metabolic plates. Of all the infectious agents we studied, *Y. pestis* appeared to be the most metabolically active, with the indicator colour appearing on the metabolic plates within an hour. However, for consistency with the other bacteria studied at DRES, the indicator colour was measured on the plate reader after 4 hours incubation and again after 16-24 hours incubation. Results in this text show the latter.

Antibiotic Sensitivity

Procedures used were as noted in the National Committee of Clinical Laboratory Studies (NCCLS) guidelines in the Clinical Microbiology Procedures Handbook (American Society for Microbiology) [8].

Results and Discussion

Culture of strains

Upon plating the strains of *Yersinia pestis* onto various media, this bacterium was very hardy, growing readily on trypticase soy agar (TSA) and *Yersinia* selective agar (CIN) medium with supplement. The latter was of concern to us because it has inhibiting components in the medium (e.g. the detergent bile salt and the dye crystal violet) and antibiotics (e.g. cefsulodin, irgasan and novobiocin) in the supplement. However, in comparison to growth on TSA, the strains appeared to grow better on the *Yersinia* selective medium with supplement. Although colonies on both agars were similar, being smooth and waxy in appearance, colonies on the *Yersinia* medium were red due to the uptake of neutral red and crystal violet dyes in the medium.

Biolog™ Metabolic Identification Profiles

The metabolites of the Biolog™ GN-2 (GN stands for Gram-negative) microtitre plate as provided by Biolog Inc. are shown in Table 1. Table 2 gives a summation of the metabolites used by the 3 strains of *Y. pestis*. Individual profiles of each of the strains are shown in Annex A. The profiles of each strain show a clear similarity, although we may find greater variability once we test additional strains. A comparison of our *Y. pestis* data with the Biolog™ data base on *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (data not shown) shows a clear distinction between these organisms.

Table 1: Substrate locations on Biolog GP-2 Plate			
Well	***Substrate***	***Well***	***Substrate***
A1	Water	E1	p-hydroxy phenylacetic acid
A2	α-cyclodextrin	E2	Itaconic acid
A3	Dextrin	E3	α-keto butyric acid
A4	Glycogen	E4	α- keto glutaric acid
A5	Tween 40	E5	α-keto valeric acid
A6	Tween 80	E6	D,L-lactic acid
A7	N-acetyl-D-galactosamine	E7	Malonic acid
A8	N-acetyl-D-glucosamine	E8	Propionic acid
A9	Adonitol	E9	Quinic acid
A10	L-arabinose	E10	D-saccharic acid
A11	D-arabitol	E11	Sebacic acid
A12	Cellobiose	E12	Succinic acid
B1	i-erythritol	F1	Bromo succinic acid
B2	D-fructose	F2	Succinamic acid
B3	L-fucose	F3	Glucuronamide
B4	D-galactose	F4	Alaninamide
B5	Gentiobiose	F5	D-alanine
B6	α-D-glucose	F6	L-alanine
B7	m-inositol	F7	L-alanyl-glycine
B8	α-D-lactose	F8	L-asparagine
B9	Lactulose	F9	L-aspartic
B10	Maltose	F10	L-glutamic
B11	D-mannitol	F11	Glycyl-L-aspartic acid
B12	D-manose	F12	Glycyl-L-glutamic acid
C1	D-melibiose	G1	L-histidine
C2	β-methyl D-glucose	G2	Hydroxy L-proline
C3	D-psicose	G3	L-leucine
C4	D-raffinose	G4	L-ornithine
C5	L-rhamnose	G5	L-phenylalanine
C6	D-sorbitol	G6	L-proline
C7	Sucrose	G7	L-pyroglutamic acid
C8	D-trehalose	G8	D-serine
C9	Turanose	G9	L-serine
C10	Xylitol	G10	L-threonine
C11	Methyl pyruvate	G11	D,L-carnitine
C12	Mono-methyl succinate	G12	γ-amino butyric acid
D1	Acetic acid	H1	Urocanic acid
D2	Cis-aconitic acid	H2	Inosine
D3	Citric acid	H3	Uridine
D4	Formic acid	H4	Thymidine
D5	D-galactonic acid lactone	H5	Phenyl ethylamine
D6	D-galacturonic acid	H6	Putresine
D7	D-gluconic acid	H7	2-amino ethanol
D8	D-glucosaminic acid	H8	2,3-butanediol
D9	D-glucuronic acid	H9	Glycerol
D10	α-hydroxy butyric acid	H10	D,L-α-glycerol phosphate
D11	β-hydroxy butyric acid	H11	Glucose-1-phosphate
D12	γ-hydroxybutyric acid	H12	Glucose-6-phosphate

Table 2: Summary of *Yersinia pestis* Biolog Metabolic Profile

A1	A2		A4	A5		A7		A9	A10		A12 (2.5/3)
B1		B3	B4 (2.5/3)	B5		B7	B8	B9			
C1	C2 (2/3)			C5	C6	C7		C9 (2.5/3)	C10		C12
	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5		E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6 (2/3)	F7 (2/3)	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6 (2.5/3)	G7	G8		G10	G11	G12
H1	H2		H4	H5	H6	H7		H9	H10	H11	H12

In this figure, the wells shaded red were positive for all three strains of *Yersinia pestis* tested. Wells shaded yellow were positive for only 2 strains, while wells shaded green were positive in only one. Unshaded wells were negative for all three strains. The numbers in parentheses indicate the number of strains that tested positive in that well. For the purposes of this figure, borderline reactions are indicated by a value of 0.5. (eg. If 2 strains were positive for a certain well and 1 strain was borderline, then the number in parentheses would be 2.5)

Antibiotic Sensitivities

Table 3 shows a summation chart on the antibiotic sensitivities for the 3 strains of *Y. pestis* tested. Although the number of strains is low and there is a requirement to acquire more strains to validate the results, there are still obvious patterns of sensitivities and resistance that surface. All three *Y. pestis* strains tested were resistant to lincosamide (e.g. clindamycin), macrolide (e.g. erythromycin), rifampin and vancomycin, polymyxin B and sulfonamides such as sulfisoxazole. Similar results have been reported for other plague strains [9]. We were surprised to see that *Y. pestis* strain GB, in our tests, was resistant to doxycycline. This is in contrast to other reports that *Y. pestis* GB strain is sensitive to doxycycline as determined by animal challenge studies in mice [10] and is recommended chemotherapy plague in general [1]. We will be revisiting this issue to determine why this result occurred. All strains tested were sensitive to the aminoglycosides, most of the beta lactams (aside from oxacillin), most of the quinolones (aside from bacitracin) and most of the tetracyclines. It is our intention to acquire and test additional strains of *Y. pestis*.

Table 3: Antibiotic sensitivities for *Yersinia pestis* strains

ANTIBIOTIC FAMILY	ANTIBIOTIC	CODE AND AMOUNT (ug)	RESISTANCE (R) or SENSITIVITIES (S)*
Aminoglycoside	Amikacin	AN30	S (0.5/3)
	Gentamycin	GM10	S (0/3)
	Kanomycin	K30	S (0/3)
	Neomycin	N30	ND
	Streptomycin	S10	S (1/3)
	Tobramycin	TM10	S (0/3)
Beta Lactams & Penicillins	Ampicillin	AM10	S (0/3)
	Carbenicillin	CB100	S (0/3)
	Piperacillin	PIP100	S (0/3)
	Ticaracillin	TIC75	S (0/3)
	Oxacillin	OX1	R (2/3)
Cephalosporins & Cephans	Cefotaxime	CTX30	S
	Ceflazidime	CAZ30	S (0/3)
	Cethalopin	CR30	S (0/3)
Lincosamide	Clindamycin	CC2	R (3/3)
Macrolide	Erythromycin	E15	R (3/3)
Others	Chloramphenicol	C30	S (0/3)
	Colistin	CL10	R (3/3)
	Nitrofurantoin	FD300	S (0/3)
	Novobiocin	NB30	ND
	Rifampin	RA5	R (2.5/3)
	Vancomycin	VA30	R (3/3)
Peptide	Bacitracin	B10 units	R (3/3)
Polypeptides	Polymixin B	PB300	R (3/3)
Quinolones	Ciprofloxacin	CIP5	S (0/3)
	Nalidixic acid	NA30	S (0.5/3)
	Norfloxacin	NOR10	S (0/3)
Sulfonamide	Sulfisoxazole	G300	R (3/3)
Sulfonamides	Trimethoprim	SxT25	ND
Tetracyclines	Doxycycline	DO30	S (1/3)
	Tetracycline	TE30	S (0/3)

*For resistance or sensitivities to the various antibiotics, a value of 1 is given to each strain that is resistant, 0.5 if it is intermediate and 0 if it is sensitive, and this number is divided by the number of strains. A value of 1/3 may mean that 1 of 3 strains is resistant to the given antibiotic or that 2 strains were intermediate in their resistance.

Defence Reference Center for Infectious Agents (DRCIA)

This paper is a small part of a larger overall study in which DRES is characterizing all the bacterial Risk Group 3 agents being used in our research program. Characterization has begun on *Y. pestis* (this study), *Bacillus anthracis* [11], as well as *Brucella spp.*, *Burkholderia spp.* and *Francisella tularensis* (manuscripts in preparation). Although DRES does not have a mandate or capability, at present, to carry out diagnostic identification work, DRES conducts research into developing identification techniques (eg. gene probes, immunochromatographic assays, ELISA's, etc) for potential BW agents and evaluating other potential identification tools that could be adapted to serve CF needs (eg. Biolog metabolic identification, antibiotic resistance profiling). While some of this work can be carried out in Level 2 laboratories using simulants or killed materials, it is vital that this research extend into working with live threat agents. This can only be done if DRES maintains a reference collection of potential threat agents which can be used to fully test the new identification techniques.

DRES has already participated in NATO identification training exercises in 1999 and 2000 (manuscripts in preparation) in which killed pathogen samples were supplied to participating NATO labs as coded unknown samples for identification. Provisional and some Confirmed identifications were successfully carried out in these exercises. The NATO criteria for the identification of biological agents is broken down into 3 groupings: Provisional, Confirmed and Unambiguous. "Provisional Identification" is achieved when: a) the presence of a unique antigen for the microbial agent in question is demonstrated by a positive reaction with a specific antibody **or** b) when the presence of a unique nucleic acid sequence for the microbial agent in question is demonstrated by a positive reaction with a specific gene probe **or** c) when a positive response is indicated by *in vitro* culture/biochemical analysis tests. "Confirmed Identification" is achieved with a combination of positive responses in any two of the provisional identification criteria is met. "Unambiguous Identification" requires a positive response from all three of the provisional identification criteria **and** that the disease properties of the microbial agent are confirmed in an accepted animal model [12]. DRES will participate in the next exercise in 2001 and has also requested that a parallel set of live samples be sent to us in addition to the killed materials to allow DRES to gain experience with *in vitro* culture/biochemical analysis of live unknowns. These exercises have shown that DRES has a limited identification capability, but also identified areas where an expanded effort will be needed if DRES is ever required to fulfill a true rapid BW identification mandate.

The proposed DRCIA would formalize and expand upon a number of existing DRES resources and capabilities. It's primary purpose would be to serve as a culture collection for defence research purposes. International regulations are becoming more stringent making it far more difficult to obtain Risk Group 3 cultures than in the past. Maintaining and expanding the culture collection at DRES would ensure future access to this material, a resource which will be of value in future protection and identification studies.

With the reference collection in place, the cultures could be characterized to establish an identification database of information for the threat agents of interest. This study is a step in that direction. As new cultures are obtained, or new identification technologies developed, the database will expand. The database will also include information on disinfectant sensitivities and antibotic sensitivity profiles that we are gathering as part of our Safety SOP's. Other

areas that would likely fall under the DRCIA mandate include such things as: a) sampling kits and protocols; b) forensic handling of evidence; c) sample preparation; d) sample processing protocols. DRES has direct experience in some of these areas (a,b) and is involved in international collaboration (AG-47 – BTWC related analytical methodologies) dealing with the other issues (c,d).

DRCIA could serve as a biological agent identification laboratory for the CF, much as DRES already formally serves as a chemical agent identification laboratory. In the event of BW use against CF personnel, or terrorist use of BW agents in Canada, there will be a need for biological identification. If the concept of DRCIA is accepted and adequate resources provided, DRES could expand upon its limited identification capabilities to be able to fulfill the new mandate of rapid BW agent identification when needed.

Conclusion

Biolog™ metabolic profiles were determined for each of the 3 *Yersinia pestis* strains held at DRES. These profiles, together with those for other Risk Group 3 agents at DRES, could serve as the basis for the proposed DRCIA identification capabilities. Antibiotic sensitivity profiles confirm that all *Y. pestis* strains at DRES are sensitive to a variety of antibiotics (including Ciprofloxacin), although we were surprised to find that *Yersinia pestis* GB strain was resistant to Doxycyclin in contrast to other published reports. It is our intention to aquire additional strains of *Y. pestis* (and other Risk Group 3 agents) to expand our database profile.

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Annex - Biolog™ metabolic identification profiles of all *Yersinia pestis* strains tested

Yersinia pestis C12

A1	A2	**A3 +**	A4	A5	A6	A7	**A8 +**	A9	A10	**A11 ±**	**A12 ±**
B1	**B2 +**	B3	**B4 ±**	B5	**B6 +**	B7	B8	B9	**B10 +**	**B11 +**	**B12 +**
C1	**C2 +**	**C3 +**	C4	C5	C6	C7	**C8 +**	**C9±**	C10	**C11 +**	C12
D1 +	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	**E6 +**	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	**G6±**	G7	G8	**G9 +**	G10	G11	G12
H1	H2	**H3 +**	H4	H5	H6	H7	H8	H9	H10	H11	H12

Yersinia pestis C092

A1	A2	**A3 +**	A4	A5	A6	A7	**A8 +**	A9	A10	**A11 +**	**A12 +**
B1	**B2 +**	B3	**B4 +**	B5	**B6 +**	B7	B8	B9	**B10 +**	**B11 +**	**B12 +**
C1	**C2 +**	**C3 +**	C4	C5	C6	C7	C8 +	C9 +	C10	**C11 +**	C12
D1 +	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	**E6 +**	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	**F6+**	**F7 +**	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	**G6 +**	G7	G8	**G9 +**	G10	G11	G12
H1	H2	**H3 +**	H4	H5	H6	H7	H8	H9	H10	H11	H12

Yersinia pestis GB

A1	A2	**A3 +**	A4	A5	**A6 ±**	A7	**A8 +**	A9	A10	**A11 +**	**A12 +**
B1	**B2 +**	B3	B4	B5	**B6 +**	B7	B8	B9	**B10 +**	**B11 +**	**B12 +**
C1	**C2 +**	**C3 +**	C4	C5	C6	C7	**C8 +**	**C9 +**	C10	**C11 +**	C12
D1 +	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	**E6 +**	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	**F6 +**	**F7 +**	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	**G6 +**	G7	G8	**G9 +**	G10	G11	G12
H1	H2	**H3 +**	H4	H5	H6	H7	H8	H9	H10	H11	H12

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14. ABSTRACT

(U) We have characterized 3 strains of Yersinia pestis currently held in the culture collection at DRES by colonial morphology, antibiotic sensitivity and Biolog™ metabolic identification profiles On agar medium, colonies were round and waxy, resembling other Gram-negative cells such as Escherichia coli. Cultures grew better on Yersinia selective media than on trypticase soy agar or nutrient agar. Antibiotic sensitivities showed that the 3 strains were sensitive to aminoglycosides, the cephalosporins/ cephams, most of the beta lactams/penicillins (e g. ampicillin) and quinolones (e g. ciprofloxacin). These strains were resistant to lincosamide (e.g. clindamycin), macrolides (e.g erthromycin, colisitin, rifampin and vancomycin) polymyxin B, sulfonamide (e.g. sulfisoxazole) and Yersinia pestis strain GB was resistant to doxycycline.

15. KEYWORDS, DESCRIPTORS or IDENTIFIERS

(U) Yersinia pestis, plague, identification, Biolog